REMARKS/ARGUMENTS

Claims 1-5 and 7-9 are pending.

Claims 1, 3-5 and 8-9 have been amended.

Claims 6 and 10-29 have been cancelled.

Support for the amendments is found in the claims and specification, as originally filed. Specifically, support for the limitation "the EphA2 protein" of amended claims 1, 3-4, and 9 can be found at least on page 1, lines 1-3; for the limitation "immunogenic peptide" of amended claims 4-5 can be found at least on page 1, lines 5-12; for the limitation "the binding affinity and/or the stability" of amended claim 3 can be found at least on pages 4, line 21, through page 5, line 35 and in the Examples on pages 8-10; for the limitation "at least one epitope derived from the EphA2 protein or from one or more other antigens" of amended claim 8 can be found at least on page 6, line 35, through page 7, line 19; for the limitation "composition comprises a chimeric polypeptide comprising at least one peptide selected from the group consisting of at least one copy of the immunogenic peptide constituting a T epitope presented by MHC I, wherein said immunogenic peptide consists of a fragment of 8 to 11 consecutive amino acids of the EphA2 peptide, and at least one copy of another immunogenic peptide derived from the EphA2 protein or one or more other antigens" of amended claim 9 can be found at least on page 7, lines 8-19. No new matter is believed to have been added.

Applicants thank the Examiner for clarifying the objections to the Oath and Declaration and the first page of the specification during discussion with the undersigned on July 17, 2007. Applicants submit that handwritten notations on the Oath and Declaration and the first page of the specification were done by the Patent and Trademark Office after the documents had been filed. Applicants re-submit with this paper a copy of the first page of the originally filed specification as suggested by to the Examiner.

The specification has been amended to disable hyperlinks and to place descriptions of drawing that can be found throughout the specification in the section "Brief description of drawings." Support for the amendments can be found on page 2, line 28, pages 13-16, and page 18 of the originally filed specification.

Figure 1 now identifies the sequence by SEQ ID NO: 1, as suggested by the Examiner. Support for the amendment can be found on page 2, line 28, of the originally filed specification.

Claims 1-5 and 7-9 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite.

Claim 3 now recites "Eck tyrosin kinase receptor (EphA2)" and claims 1-5 and 7-9 to recite "EphA2 protein."

Claims 4-5 have been amended to clarify that "said peptide" is "said immunogenic peptide."

Claim 3 has been amended to clarify that the immunogenic peptide is derived from a peptide consisting of a fragment of 8-11 amino acids of the EphA2 protein, by substituting at least one amino acid of the peptide which [substitution] increases the binding affinity and/or the stability of the peptide [e.g., derived immunogenic peptide has increased affinity and stability compared to the unsubstituted peptide]. This is supported by the specification on page 4, line 21, through page 6, line 34, and page 9, line 10, through page 11, line 15.

Claim 3 has been amended to also recite that substitution of amino acids of the peptide increases the binding affinity and/or stability of the peptide. The specification clearly discloses that the affinity of a peptide for an MHC I molecule is most commonly defined with respect to a reference peptide in the form of relative affinity which is defined as a ratio of the concentration of the tested and reference peptides (page 4, lines 32, through page 5, line 29;

page 6, lines 2-34; page 9, lines10 through page 10, line 33). The stability of the complex is defined by the DC_{50} (page 5, lines 7-13; page 10, lines 11-33). Applicants submit that the term "increases" affinity and stability is clear in view of the disclosure.

Claim 8 has been amended to clarify that a multiepitope composition also comprises at least one epitope derived from the EphA2 protein or from one or more other antigens" (page 6, line 35, through page 7, line 19).

Claim 9 has been amended to clarify that the "composition comprises a chimeric polypeptide comprising at least one peptide selected from the group consisting of at least one copy of the immunogenic peptide constituting a T epitope presented by MHC I, wherein said immunogenic peptide consists of a fragment of 8 to 11 consecutive amino acids of the EphA2 protein, and at least one copy of another immunogenic peptide derived from the EphA2 protein or one or more other antigens" (page 7, lines 8-19).

Applicants request that the rejections be withdrawn.

Claims 8 and 9 are rejected under 35 U.S.C. 112, first paragraph, for lack of written description.

Claim 8 has been amended to recite that the composition is a multiepitope composition which also comprises at least one peptide derived from the EphA2 protein or from one or more other antigens (page 6, line 35, through page 7, line 19).

Claim 9 has been amended to recite that the composition "composition comprises a chimeric polypeptide comprising at least one peptide selected from the group consisting of at least one copy of the immunogenic peptide constituting a T epitope presented by MHC I, wherein said immunogenic peptide consists of a fragment of 8 to 11 consecutive amino acids of the EphA2 protein, and at least one copy of another immunogenic peptide derived from the EphA2 protein or one or more other antigens" (page 7, lines 8-19).

Applicants request that the rejections be withdrawn.

Claims 1-5 and 7-9 are rejected under 35 U.S.C. 112, first paragraph, for lack of enablement.

The rejection is unsustainable because even if the art known before the filing date of the application supports "unpredictability" of generating immunogenic peptides, the specification discloses a different method of producing an immunogenic peptide, *i.e.*, linking genomics to immunotherapy by reverse immunology, and, therefore, the specification does provide guidance on how to generate an immunogenic peptide constituting a T epitope presented by MHC I consisting of a fragment of the EphA2 antigen.

The Examiner asserted that claims as not enabled for any EphA2-derived peptide producing an immunogenic response in any cancer patient. However, the Examiner appears to indicate that the claims are enabled for specific peptides and the specific cancer in the specification (e.g., melanoma, prostate cancer, lung cancer, sarcoma, colon cancer, renal carcinoma). The rejection states that there is a poor correspondence between actual and predicted binding when using predictive computer algorithms; increasing binding activity does not consistently and reproducibly relate to a peptide epitope's immunogenicity; other factors are important for stimulating an effective immune response; and one cannot expand the teaching of the specification because it does not provide examples of how to use the claimed peptide for active immunotherapy *in vivo* (pages 11-20 of the Official Action). The Examiner cites a number of references to support the assertion that the art of synthesizing functional equivalents of naturally occurring proteins is very unpredictable, particularly, in terms of *in vivo* uses.

Reverse immunology (extensively exemplified in the specification), compared to, classical antigen discovery approaches, is superior and allows discovery of immunogenic peptides for large variety of tumor antigens.

As stated in the submitted Declaration of Dr. Kosmatopoulos, to be considered as an immunogenic T epitope presented by MHC I, a peptide must fulfill several conditions, which are listed in paragraph 3 of the Declaration:

Therefore one can conclude that:

i) All peptides transported to the endoplasmic reticulum (ER) are not epitopes. Only peptides binding to HLA preferably with high affinity, can efficiently be presented at the cell surface.

ii) All peptides that have a high affinity for HLA are not epitopes. To be epitopes they have to be produced by the proteasome and transported to the ER.

iii) All epitopes are not immunogenic, especially those that are expressed at the surface of normal cells. For an epitope be immunogenic, it is necessary that the immune system is not tolerant to it.

To identify immunogenic epitopes, candidates for tumor vaccinations, must take into account all the above-described parameters.

Using algorithms predicting HLA affinity of peptides might allow the identification of peptides with high HLA affinity but this method suffers from several limitations known by one skilled in the art as confirmed by the prior art (See Schirle et al. and Anderson et al., cited by the Examiner) and Dr. Kosmatopoulos (See paragraph 4 of the Declaration).

In contrast, the reverse immunology that is a multistep process for immunogenic epitope identification, allows for:

i) selection of peptides with high affinity for HLA using affinity prediction algorithms,

ii) selection among high HLA affinity peptides of those that are immunogenic both *in vivo* in appropriate HLA transgenic mouse models and *in vitro* in human T lymphocytes (for instance see Example 4, page 16 of the specification), and

iii) selection among all immunogenic peptides of those that are efficiently produced by the proteasome, transported to the ER and finally presented on the surface of tumor cells to be recognized by and stimulate specific T cells.

The reverse immunology approach has been widely used for the identification of immunogenic tumor epitopes presented by different HLA molecules and is well known for one skilled in the art, as shown by the following enclosed articles:

Kawashima, I., V. Tsai, et al. (1999). "Identification of HLA-A3-restricted cytotoxic T lymphocyte epitopes from carcinoembryonic antigen and HER-2/neu by primary in vitro immunization with peptide-pulsed dendritic cells." Cancer Res 59(2): 431-5.

Tahara, K., K. Takesako, et al. (1999). "Identification of a MAGE-2-encoded human leukocyte antigen-A24-binding synthetic peptide that induces specific antitumor cytotoxic T lymphocytes." Clin Cancer Res 5(8): 2236-41.

Lu, J. and E. Celis (2000). "Use of two predictive algorithms of the world wide web for the identification of tumor-reactive T-cell epitopes." Cancer Res 60(18): 5223-7.

Vonderheide, R. H., K. S. Anderson, et al. (2001). "Characterization of HLA-A3-restricted cytotoxic T lymphocytes reactive against the widely expressed tumor antigen telomerase." Clin Cancer Res 7(11): 3343-8.

In the paragraph A 2), pages 16 and 17, the Examiner cites the article of Ezzell, dated 1995, which gives a pessimistic review of cancer vaccines. However, the Applicant submits that recent insights in the field of tumor immunology are breathing new life into cancer vaccines. For example, vaccines targeting simultaneously different components (innate,

adaptive B and T immunity) of the immune system are expected to have a significant clinical efficacy, as shown in the enclosed publications:

Nemunaitis and Nemunaitis, *Expert Opinion on Biological Therapy*, 2007, 7, 89-102 (see Expert Opinion)

Gilboa, *Nature Rev. Cancer*, 2004, 4, 401-411 (see Summary)

O'Mahony *et al.*, *J. Clin. Oncol.*, 2005, 23, 9022-9028 (see Conclusion)

Further, concerning the assertion that "*no one is very optimistic that a single peptide will trigger an immune response strong enough to eradicate tumors or even prevent the later growth of micrometastases* ..." (sentence bridging pages 16 and 17), the Applicant would like to submit that tumor vaccines containing a single peptide and inducing a monospecific immune response such as GV1001 and Stimuvax (L-BLP-25), have successfully reached the last step of their clinical development, as shown for example in part 2 of *Expert Opinion on Biological Therapy*, 2007, 7, 89-102.

In Table 1 below, the Applicant submits a list of vaccines based on single peptides and which are currently in phase II or III of clinical development:

Vaccine	Company	Phase
GV1001	Pharmexa	III
Stimuvax (BLP-25)	Merck/Biomira	III
CYT004-MelQbG10	Cytos	II
Avicine	AviBiopharma	II
Prime Boost Melanoma Therapy	Oxxon	II
CMLVAX100	Breakthrough Thera	II

Table 1: Single peptide vaccines currently in clinical development

Thus, the claimed immunogenic peptide can be predictably obtained by using reverse immunology described in the specification and that this method has been successfully used by scientists to obtain various immunogenic peptides that were used in preclinical vaccination studies.

Applicants request that the rejections be withdrawn.

Claims 1, 2, and 7 are rejected under 35 U.S.C. 103(a) over Powell, US 2007/0031882 and Parker, J. Immunol., 152:163-175 (1994).

The rule 132 Declaration from Dr. Kosmatopoulos clearly explains why it was not obvious to obtain T-cell epitopes from EphA2.

Parker *et al.* (J. Immunol., 152:163, 1994) teaches methods (BIMAS program) to select peptides potentially capable of binding to HLA-A*0201 with a high affinity.

Although the program disclosed by Parker et al. could be applied to the EphA2 sequence to determine whether some fragments of this proteins could bind to HLA-A*0201 with a high affinity, the skilled artisan could not know, before doing so, if the EphA2 amino acids sequence comprises any fragment (naturally produced by the proteasome or not) binding to HLA-A*0201 with a high affinity.

The present specification discloses a different method of producing an immunogenic peptide, *i.e.*, linking genomics to immunotherapy by reverse immunology, and describes how to generate an immunogenic peptide constituting a T epitope presented by MHC I consisting of a fragment of the EphA2 protein.

Moreover, even if a skilled artisan applied the BIMAS program and found a high HLA affinity peptide corresponding to a fragment of EphA2, this would not have meant that said peptide was an epitope, and Parker *et al.* do not provide any information as to how to determine if a high HLA affinity peptide is an epitope. Indeed, a high HLA affinity peptide (property (ii) mentioned above) which consists of a fragment of 8 to 11 consecutive amino acids of a protein is not necessarily an epitope, since it is not obvious that this peptide is naturally produced by the proteasome digestion of the protein from which it is derived. If it is not produced by the proteasome, then it cannot be presented by the MHC, and it can therefore not constitute a CTL epitope. Kessler *et al.* (J. Exp. Med., 193:73-88, 2001, enclosed)

reported that only 21% of high affinity HLA-A*0201 binding peptides were found to be efficiently generated by proteasome and presented by the said HLA molecule.

In addition to the arguments developed by Dr. Kosmatopoulos, we would like to submit to the Examiner the following remarks/arguments.

Powell et al. teach that EphA2 or a fragment thereof can be used as immunogen to generate antibodies binding EphA2, that is a process involving *B cell* activation. However, Powell et al. fail to teach or suggest an immunogenic peptide constituting a *T epitope* presented by MHC I derived from EphA2.

A critical difference exists between B cell and T cell activation. Indeed, B cells recognize their cognate antigen in their native tridimensional form in the blood or lymph using their B cell receptor, while T cells recognize their cognate antigen in a processed form (linear peptide) as stated in paragraph 3 of the Dr. Kosmatopoulos' Declaration.

Although Parker et al. disclose methods for selecting peptides potentially capable of binding to HLA-A*0201 with high affinity, the skilled artisan would not have found any motivation to combine both cited documents and to try to develop immunotherapy targeting EphA2, that is a process involving T cells.

Applicants request that the rejections be withdrawn.

Claims 1, 2, and 7 are rejected under 35 U.S.C. 103(a) over Lindberg, Mol. Cell Biol., 10(12):6316-6324 (1990), Parker, J. Immunol., 152:163-175, and Renkvist, Cancer Immunol. Immunother, 50:3-15 (2001).

Lindberg *et al.* teaches the cloning and the amino acid sequence of EphA2 and its expression by normal epithelial cells. Lindberg *et al.* also suggest that the gene encoding EphA2 might be a proto-oncogene, but this article is silent about the potential immunogenicity of EphA2.

Lindberg et al. suggest that *eck* might be a putative proto-oncogene. They also teach that NIH-3T3 cells artificially expressing the *eph* gene (and not *eck*) to a high level acquire tumorigenic ability in nude mice, suggesting that the PTK genes family can function as oncogenes. However, Lindberg et al. fail to teach or suggest that *eck* gene (and even *eph* gene) is over-expressed in tumors, or is expressed in tumors at higher levels than in tissues in which it is normally expressed.

The disclosure by Powell is discussed above.

Renkvist *et al.* give a list of numerous tumor antigens and their HLA restricted peptides, most of them being identified by the genetic and/or biochemical method, but not the reverse immunology method used in our application.

This reference does not give any information about EphA2, and therefore, the skilled artisan could absolutely not determine, by combining the disclosures of Lindberg, Parker and Renkvist, if EphA2 contained T epitopes presented by MHC I.

As mentioned above, all epitopes are not immunogenic, especially those that are expressed at the surface of normal cells. For an epitope to be immunogenic, it is necessary that the immune system is not tolerant to it (property (iii) mentioned above).

The disclosure by Renkvist *et al.*, distinguishes several groups of tumor antigens. Certain tumor antigens result from point mutations in normal genes (Group 4), some others from translocations which create novel fusion proteins (Group 6), whereas some others are encoded by genes which are normally not expressed in non-tumor cells, or only in certain immuno-protected tissues (such as the cancer/testis antigens of Group 1). It is not surprising that these antigens can induce a CTL reaction, provided they are efficiently processed by the proteasome, since the immune system never had the opportunity to become tolerant to them. Renkvist et al. teach several groups of tumor antigens recognized by T cells, but fail to teach or suggest tumor antigens derived from EphA2.

To the contrary, EphA2 is expressed in normal epithelial cells, as disclosed by Lindberg *et al.* EphA2 is expressed especially in kidney and lung. The skilled artisan knows that the education of T cells (which takes place mainly in the thymus) leads to the elimination of auto-reactive cells, *i.e.*, cells which recognize a self antigen.

Renkvist *et al.* teach that some widely expressed tumor antigens have also been detected in normal tissues, and they classify these in Group 3 (page 4, right column, last paragraph). To explain this phenomenon, they hypothesize that "*many epitopes expressed on normal tissues are below the threshold level for T-cell recognition, while their overexpression in tumor cells can trigger an anticancer response even by breaking a previously established tolerance*". However, there is nothing in the disclosures of either Lindberg, Parker, or Renkvist, which suggests that the expression level of EphA2 in normal cells is below the T-cell recognition threshold, and none of these documents mentions that it is strongly overexpressed in tumor cells.

Hence, the skilled artisan could not anticipate that putative EphA2 epitopes would be immunogenic.

Therefore, the skilled artisan would not have found any motivation to combine the three cited documents, Lindberg, Renkvist and Parker to obtain T epitope peptides from EphA2.

Further, one of ordinary skill in the art would have lacked the requisite expectation of success in utilizing the methods taught by Parker et al. since they do not provide any information as to how to determine if a high HLA affinity peptide is a useful immunogenic T epitope (see arguments presented to overcome the Enablement rejection).

Applicants request that the rejections be withdrawn.

A Notice of Allowance for all pending claims is requested.

Respectfully submitted,

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EPHA2 ANTIGEN T EPITOPES

The present invention relates to peptides derived from the EphA2 protein and to their use in antitumor immunotherapy.

Peptide immunization or immunotherapy is a therapeutic approach which is currently the subject of a great deal of interest in the context of the prevention or treatment of cancer. The principle thereof is based on immunization with peptides which reproduce T epitopes of tumor antigens recognized by cytotoxic T lymphocytes (CTLs), which play a major role in the elimination of cancer cells expressing these antigens at their surface.

It will be recalled that CTLs do not recognize whole protein antigens, but peptide fragments thereof, presented by major histocompatibility complex (MHC) molecules expressed at the surface of various cells. It is these peptide fragments which constitute the T epitopes.

The presentation of these peptides is the result of a complex process, called "antigen processing", which involves 3 main steps:

- cytosolic degradation of the antigens by a multienzyme complex called proteasome;

- translocation of the peptides derived from this degradation into the endoplasmic reticulum (ER) by TAP transporters;

- association of these peptides with the MHC so as to form stable peptide/MHC complexes which will be exported to the cell surface.

The presentation of T epitopes at the cell surface depends in particular on the stability of the antigenic protein in the cytosol, on the sites and on the frequency of the cleavages carried out by the proteasome, on the efficiency of translocation into the ER by the TAP transporters, and on the ability of the peptides to bind to the MHC molecules and to form stable peptide/MHC complexes.